FORAGE & GRAZING LANDS

Diurnal Effects on Nutritive Value of Alley-Cropped Orchardgrass Herbage

D. M. Burner* and D. P. Belesky

ABSTRACT

Nonstructural carbohydrates, crude protein, and in vitro dry matter digestibility (IVDMD) are important measures of herbage nutritive value and can vary at seasonal and diurnal time scales in conventional agronomic systems. Our objective was to determine diurnal trends of nutritive value components in orchardgrass (*Dactylis glomerata* L.) herbage harvested daily on 1 through 15 June 2001 and 2002 from three microsites: unshaded control, loblolly pine (*Pinus taeda* L.) alleys, and shortleaf pine {(*P. echinata* Mill.) alleys at three time intervals [0500, 1100, and 1700 h, Central Standard Time (CST)]}. Diurnal responses were defined as a regression response or change in mean ($P \leq 0.10$) with time. Orchardgrass herbage exhibited temporal change in total nonstructural carbohydrate (TNC), water-soluble carbohydrate (WSC), starch, and IVDMD. At any given sampling time, TNC was greater ($P \leq 0.05$) in control than in pine microsites. Diurnal response of TNC differed among microsites ($P = 0.10$), with more rapid TNC change in the control and shortleaf pine than loblolly pine ($P < 0.01$). The differential response of TNC in pine microsites compared with the control was attributed to an altered amount and temporal distribution of solar irradiance. Diurnal responses of WSC, starch, and IVDMD were unaffected by microsite. Herbage levels of crude protein and IVDMD were greater, but yield and nonstructural carbohydrates were lower in pine microsites compared with the unshaded control. We conclude that diurnal change in herbage nutritive value tends to be buffered against irradiance constraints in alley crop environments.

A KEY COMPONENT linking canopy management and livestock grazing behavior is the nonstructural carbohydrate composition of herbage irrespective of growth environment. Nonstructural carbohydrates influence plant growth and regrowth, development, reproduction, and survival through a complex process of dynamic source–sink relationships (Fulkerson and Donaghy, 2001). Herbage concentrations of nonstructural carbohydrates can vary along short temporal (hourly or diurnal) gradients (Ciavarella et al., 2000b; Holt and Hilst, 1969), typically increasing during the day when rates of cellular biosynthesis exceed respiration, and decreasing at night. Livestock prefer pasture herbage with higher vs. lower concentration of nonstructural carbohydrate (Ciavarella et al., 2000a). Similarly, afternoon-cut hay was preferred to morning-cut hay by livestock (Fisher et al., 1999; Kothmann, 1966; Mayland et al., 2000) and could improve milk (Mayland and Shewmaker, 2000) or liveweight (Lee et al., 2001) production gains.

Herbage nutritive value is influenced by conditions including partial shade (Kephart and Buxton, 1993), resulting in altered morphology (e.g., increased leaf area and decreased specific leaf weight and tillering; Devkota and Kemp, 1998–1999) and physiology or biochemistry (e.g., nitrate and nonstructural carbohydrate accumulation). Microsite conditions such as solar irradiance (Chazdon and Pearcy, 1991; Reifsnyder et al., 1971), soil moisture (Jose et al., 2000), and soil temperature (Morecroft et al., 1998), may differ between agroforestry and conventional production environments and cause differences in herbage yield (Burner, 2003; Burner and Brauer, 2003). Since nonstructural carbohydrates vary diurnally and concentrations in herbage are linked to grazing behavior and canopy management, understanding the diurnal responses of forages to shading is warranted for optimal management of silvopastoral systems. Our objective was to determine diurnal patterns of selected nutritive value components of alley-cropped orchardgrass herbage.

MATERIALS AND METHODS

The study site was described in detail by Burner (2003). Briefly, loblolly or shortleaf pine was planted in north–south orientation in spring 1992 in four-row blocks 30 m long, at 1.2 m intervals within rows and 4.9 m between rows at Booneville, AR (35°05′N, 93°59′W, 152 m above sea level). The soil was a Linker fine, sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Hapludults). 'Potomac' orchardgrass was established in pine alleys and in control (unshaded) plots in September 1999. Plots were fertilized to supply 56 kg ha^{-1} each of N, P, and K after each spring and fall harvest, and in late winter. Plots were clipped to 3-cm stubble in April 2001 and 5-cm stubble in April 2002.

Solarimetry data collected at the site on 1 through 15 June 2000 and 2001 was used to characterize microsites during the same growth interval in 2001 and 2002. Solarimetry data were not collected in 2002. Solar irradiance was monitored with a Delta-T (Delta-T Devices Ltd., Cambridge, UK)[1] system consisting of 10 TSL tube solarimeters (spectral response 0.35 to 2.5 μm) connected to a DL2e logger. Solarimeters were placed at random, perpendicular-to-tree-row orientation, about 1 m above ground surface in the middle of loblolly (five sen-

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[1] Product names and trademarks are mentioned to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA does not imply the approval of the product to the exclusion of others that may also be suitable.

Abbreviations: CST, Central Standard Time; IVDMD, in vitro dry matter digestibility; TNC, total nonstructural carbohydrate; WSC, water-soluble carbohydrate.

sors) and shortleaf-pine (four sensors) alleys. One solarimeter was located in the control for reference. Data were collected continuously at 0.5-h intervals for the 1 to 15 June growth interval in 2000 and 2001. Thus, there was one year of overlap (2001) between collection of solarimetry data and this study.

Herbage samples were collected from each microsite (control, loblolly pine, and shortleaf pine) at 0500 (sunrise), 1100, and 1700 h (CST) for the interval 1 to 15 June 2001 and 2002. Herbage within one 0.093-m^2 quadrat was clipped to a 3-cm stubble height from each microsite at each sampling time. Quadrats were sampled only once during each year of the study. Samples were predominantly orchardgrass with trace amounts of tall fescue (*Festuca arundinacea* Schreb.). Clipped samples were placed immediately on ice for transport to the laboratory, stored temporarily at −20°C, and dried in a forced-draft oven at 70°C for 48 h. Yield was converted to a kg ha^{-1} basis. Samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) to pass a 1-mm screen and further ground in a cyclone mill (Cyclotec 1093, Foss Tecator, Eden Prairie, MN) with a 1-mm screen. Ground samples were stored at −20°C. The TNCs and WSCs were determined by the method of Smith (1981) as modified by Denison et al. (1990), with (TNC) or without (WSC) amyloglucosidase, and starch concentration was determined by difference (TNC − WSC).

Samples collected in 2001 were analyzed for N by combustion (Leco FP428, Leco Corp., St. Joseph, MO), and crude protein was calculated as N (g kg^{-1}) × 6.25. Crude protein also was measured on a random subset of 33 samples in 2002 with the same method (the calibration group). The 2002 samples (calibration group and unknown samples) were analyzed by near infrared reflectance spectroscopy (NIRS) using a Bran+Luebbe near-infrared analyzer, model I/A 500 system with Sesame v. 3.1 software (Bran+Luebbe Inc., Roselle, IL) and methods similar to those described previously (Brown and Moore, 1987; Shenk et al., 1981). Calibration data for crude protein had an $R^2 = 0.95$, SE of the estimate = 1.17, and SE of cross validation = 1.22. The IVDMD was determined by the procedure of Goering and Van Soest (1970), and modified for the ANKOM Daisy II fiber analyzer #F200 (ANKOM Technology Corp., Fairport, NY).

Sample drying at 70°C for carbohydrate analysis (Smith, 1981) was perhaps high for the N and IVDMD analyses. Samples typically are dried at ≤65°C for nutritive value (Brown and Moore, 1987; Goering and Van Soest, 1970). However, Tilley and Terry (1963) found that herbage samples had comparable in vitro digestibility when freeze dried or oven dried at 40 or 100°C, and Kendall et al. (1970) dried herbage samples at 75°C for dry matter disappearance and N. Thus, we assumed that changes in N components at 70°C, if any, occurred at the same rate as possible changes in carbohydrate components (Guillard et al., 1995).

Statistical Analysis

Daily solarimetry means were analyzed by PROC MIXED (SAS Institute, 1998) to compute least squares means. PROC REG was used to generate regression equations (SAS Institute, 1998).

Yield and nutritive value were analyzed as a split plot design with 15 replicates (date). Microsite and time were main plot and split plot, respectively. Analysis of variance was conducted with the restricted maximum likelihood method in the MIXED procedure of SAS (Littell et al., 1996; SAS Institute, 1998). Degrees of freedom were calculated by Satterthwaite's approximation method (Littell et al., 1996). All effects were considered fixed for determining expected mean squares and appropriate F tests in the ANOVA except year, replication, and interactions of these effects with fixed effects. Data were analyzed by repeated measures (Littell et al., 1996) with a first-order autoregressive covariance structure [AR(1)], with time within microsite as the repeated effect. Means were separated by Fisher's protected LSD test at $P < 0.05$ (Steel and Torrie, 1980). Regression analysis was conducted for variables that had a significant ($P \leq 0.10$) microsite × time mean square. Diurnal responses were defined as either a regression response or change in mean ($P \leq 0.10$) with time. Coefficients of determination were calculated by PROC REG (SAS Institute, 1998). Graphs were produced with SigmaPlot v. 8.02 (SPSS Inc., Chicago, IL).

RESULTS

Mean solar irradiance in the 1 to 15 June 2000 and 2001 growth interval differed ($P < 0.05$) among microsites. Complex polynomial regression equations without an intercept in the model (Fig. 1) were used to describe solar irradiance within microsites. Less solar irradiance was received in shortleaf and loblolly microsites than the control at midday, and loblolly pine received less solar irradiance than shortleaf pine microsites at midday ($P < 0.05$). Mean solar irradiance at 1200 h was about 55 and 75% of the control for loblolly and shortleaf pine microsites, respectively.

Dry matter yield was greater ($P < 0.05$) in the control (3602 kg ha^{-1}) than either pine microsite (2376 and 2592 kg ha^{-1} for loblolly and shortleaf pine, respectively). We did not detect a diurnal response for yield ($P \geq 0.63$).

The microsite × time interaction was significant ($P = 0.10$) only for TNC (Table 1). The TNC increased linearly ($P \leq 0.05$) in each microsite (Fig. 2), although the trend was not significant ($P = 0.23$) in the loblolly pine microsite. At any given sampling time, TNC was greater ($P \leq 0.05$) in control than pine microsites. Further, regression coefficients were greater ($P \leq 0.01$) in control

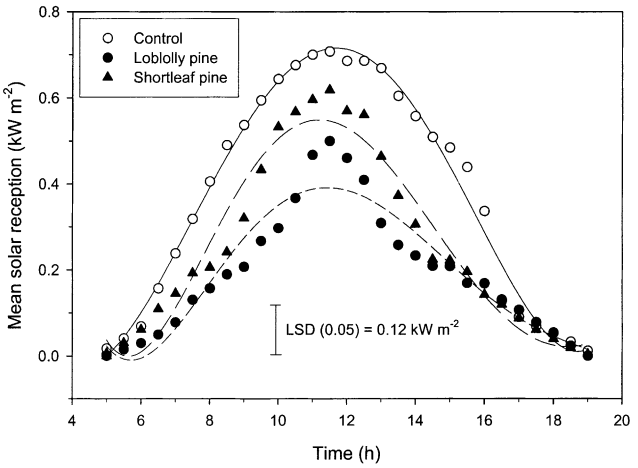

Fig. 1. Diurnal change in mean solar irradiance (λ 0.35–2.5 μm) in control (unshaded), and loblolly and shortleaf pine microsites. Data were collected from 0500 to 1930 h (CST) on 1 to 15 June 2000 and 2001. Prediction equations for solar reception were $Y = -0.045T - 0.013T^2 + 6.60 \times 10^{-3}T^3 - 5.57 \times 10^{-4}T^4 + 1.33 \times 10^{-5}T^5$, $R^2 = 0.99$; $Y = 0.024T - 0.028T^2 + 6.38 \times 10^{-3}T^3 - 4.58 \times 10^{-4}T^4 + 1.03 \times 10^{-5}T^5$, $R^2 = 0.96$; $Y = 0.018T - 0.034T^2 + 8.61 \times 10^{-3}T^3 - 6.47 \times 10^{-4}T^4 + 1.50 \times 10^{-5}T^5$, $R^2 = 0.97$ for control, loblolly pine, and shortleaf pine, respectively. T = time (h), CST. Vertical bar indicates LSD ($P = 0.05$).

Table 1. Significance of *F* values for total nonstructural carbohydrate (TNC), water-soluble carbohydrate (WSC), starch, crude protein, and in vitro dry matter digestibility (IVDMD) of orchardgrass herbage sampled on 1 through 15 June 2001 and 2002.

Source of variation	Dry matter yield	TNC	WSC	Starch	Crude protein	IVDMD
Microsite (M)	***	***	***	***	***	*
Time (T)	ns†	***	***	***	ns	*
M × T	ns	‡	ns	ns	ns	ns

* Significant at the 0.05 level.
*** Significant at the 0.001 level.
† ns, not significant.
‡ Significant at the 0.10 level.

and shortleaf pine than in loblolly pine, indicating that TNC accumulation was more rapid in microsites with greater irradiance. The WSC increased linearly (Fig. 3) and starch changed quadratically (Fig. 4) with time, but neither was differentially affected by microsite ($P \geq 0.14$). Mean WSC was higher ($P \leq 0.001$) in the control (56 g kg^{-1}) than in loblolly or shortleaf pine microsites (37 and 41 g kg^{-1}). Starch concentration nearly doubled from 0500 to 1700 h. Starch varied among microsites ($P \leq 0.05$) in the order control (6.1 g kg^{-1}) > loblolly pine (4.0 g kg^{-1}) > shortleaf pine (3.8 g kg^{-1}).

Mean crude protein varied among microsites ($P \leq 0.05$) in the order control (119 g kg^{-1}) < shortleaf pine (150 g kg^{-1}) < loblolly pine (157 g kg^{-1}), but there was no diurnal response ($P = 0.18$). Mean IVDMD was less ($P = 0.01$) in the control (655 g kg^{-1}) than in loblolly pine (667 g kg^{-1}), while IVDMD in shortleaf pine was intermediate (662 g kg^{-1}). Regression effects were not significant for IVDMD ($P = 0.24$), but mean IVDMD was greater ($P = 0.05$) at 1700 h (666 g kg^{-1}) than at 0500 h (654 g kg^{-1}).

DISCUSSION

Previous studies demonstrated that level of solar irradiance influences herbage yield (Auda et al., 1966; D.P. Belesky, 2003, unpublished data) and nutritive value

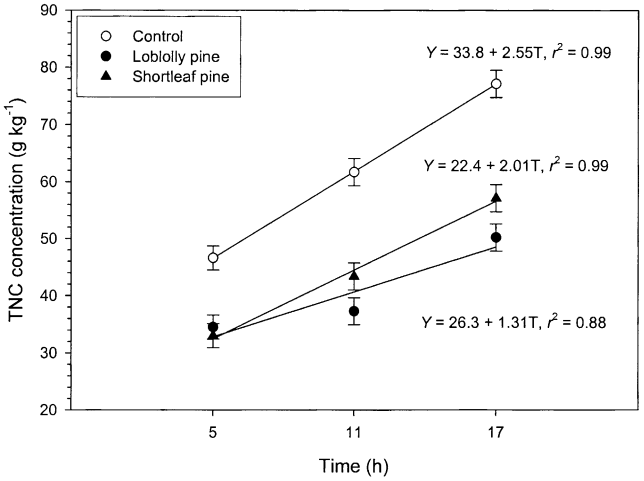

Fig. 2. Diurnal change in total nonstructural carbohydrate (TNC) in orchardgrass herbage from control (unshaded), and loblolly and shortleaf pine microsites sampled on 1 through 15 June 2001 and 2002. T = time (h), CST. The error bar at data points indicates standard error of the mean ($n = 30$).

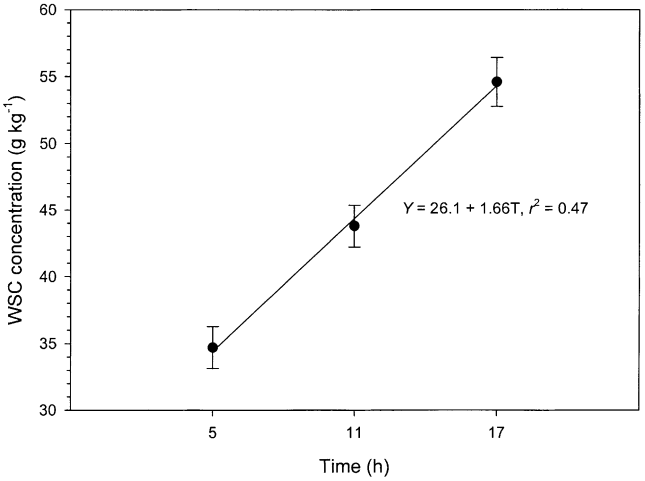

Fig. 3. Diurnal change in water-soluble carbohydrate (WSC) in orchardgrass herbage sampled on 1 through 15 June 2001 and 2002. T = time (h), CST. The error bar at data points indicates standard error of the mean ($n = 90$).

(Burner, 2003; Burner and Brauer, 2003; Ciavarella et al., 2000a; Neel et al., 2001) of cool season grasses. So, our objective was to determine diurnal patterns of selected nutritive value measures of alley-cropped orchardgrass herbage, on the assumption that microsites differed in solar irradiance.

Loblolly pine was taller (7.6 and 6.1 m, respectively) and had greater canopy cover than shortleaf pine (52 and 25%, respectively; Burner, 2003). This caused microsites to differ in amount of solar irradiance. Irradiance in shortleaf and loblolly pine microsites was comparable with that caused by 60 to 90% cloud cover or overcast sky conditions, respectively, in Missouri (McQuigg and Decker, 1958). The diurnal distribution of solar irradiance in the control was similar, but means were slightly less than values reported by Szeicz (1974) for very clear days at 52° N latitude. Similar trends probably would be observed for photosynthetically active radiation (D.M. Burner, 2003, unpublished data; Feldhake, 2001).

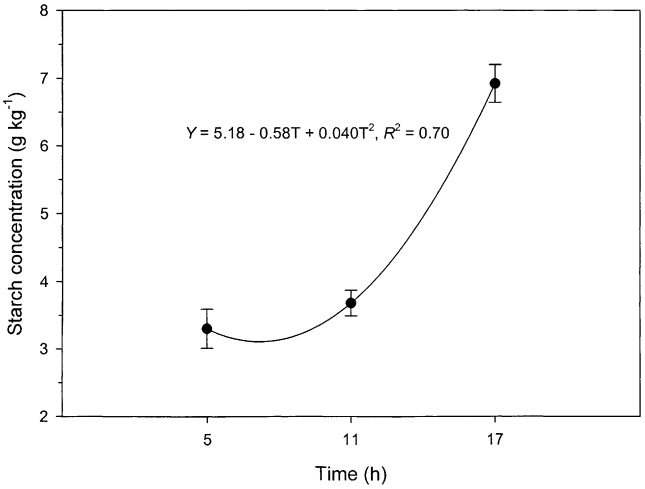

Fig. 4. Diurnal change in starch concentration in orchardgrass herbage sampled on 1 through 15 June 2001 and 2002. T = time (h), CST. The error bar at data points indicates standard error of the mean ($n = 90$).

We found diurnal responses for four nutritive value measures, TNC, WSC, starch, and IVDMD, but not crude protein or dry matter yield. The TNC concentrations were comparable with those reported for cool-season pasture herbage across a light gradient in West Virginia (Neel et al., 2001). Of measures with a diurnal response, only TNC had a microsite × time interaction. TNC increased at a faster rate in control and shortleaf pine, where light was less constraining, than in loblolly pine. Diurnal increase in herbage TNC was documented for alfalfa, *Medicago sativa* L. (Holt and Hilst, 1969; Fisher et al., 1999) and tall fescue (Fisher et al., 1999).

Across microsites, WSC increased diurnally from 35 g kg^{-1} at 0500 h to 55 g kg^{-1} at 1700 h. In the control, WSC was 42 and 69 g kg^{-1} at 0500 and 1700 h, respectively. Thus, results for the control were similar to those of Holt and Hilst (1969), who reported that mean herbage WSC of three cool season grasses increased from about 53 to 80 g kg^{-1} for the same time intervals in July. The regression equation and significance level were not reported in that study.

There was a diurnal increase in herbage starch concentration which, to our knowledge, has not been previously reported in grass-based agroforestry systems. Temperate grasses generally accumulate nonstructural carbohydrate as fructan, along with 10 to 60 g kg^{-1} starch (Mayland et al., 2000; Smith, 1971). Mean starch concentration in this study was about 5 g kg^{-1}. Holt and Hilst (1969) concluded there was a diurnal, increasing trend of starch concentration in cool-season grasses ranging from about 115 g kg^{-1} at 0600 h to 135 g kg^{-1} at 1800 h. Levels of significance were not reported, and their study was inadequate to test for a curvilinear response. There was no significant change in starch for morning- and evening-harvested tall fescue hay (Fisher et al., 1999).

Orchardgrass yield in the control was comparable with that in Arkansas variety trials (Sandage and Windham, 2000). However, herbage yield in pine microsites was about 70% of the control, demonstrating a constraint to herbage production. Similarly, Auda et al. (1966) showed that orchardgrass yields less under low light than full illumination in the greenhouse. Burner (2003) found that photosynthetically active radiation in sun flecks of loblolly pine alleys was about 10-times that in a shade patch, so yield may be reduced in proportion to amount of shaded area. However, Burner (2003) reported that orchardgrass yield was similar in loblolly pine, shortleaf pine, and control microsites. Further, Burner (2003) found that orchardgrass had greater persistence ($P \leq 0.05$) in loblolly pine (72% stand) than the control (44% stand). The discrepancy between this study and that of Burner (2003) could have been caused by different sampling dates, harvesting procedures, or microsite conditions that affected dry matter allocation, such as plant age or soil water availability.

Yield differences between control and pine microsites could be caused by soil moisture limitations in addition to light. Soil moisture was thought to influence maize (*Zea mays* L.) grain yield more than shade in hardwood alley cropping systems, on the basis of root barrier studies (Gillespie et al., 2000; Jose et al., 2000). Pine microsites have less soil moisture (D.M. Burner, 2003, unpublished data; Gillespie et al., 2000), lower soil surface temperatures (Feldhake, 2001), and less evapotranspiration (D.P. Belesky, 2003, unpublished data) than control sites. Water deficit tends to increase herbage carbohydrate concentrations when light is not limiting (Frank, 1994; Thomas and James, 1999). Relative effects of light and soil moisture constraints on production and nutritive value of perennial alley crops have not been well elucidated.

Crude protein did not vary diurnally in this study. Published reports on diurnal responses of crude protein are contradictory. Youngberg et al. (1972) reported that crude protein of alfalfa was highest between 0300 and 0600 h and declined during the day, presumably with dilution by nonstructural carbohydrates. Conversely, sheep (*Ovis aries* L.) masticate had more ($P < 0.05$) crude protein in afternoon than morning on summer sagebrush-grass range (Kothmann, 1966), while cattle (*Bos taurus* L.) masticate from summer rangeland herbage in morning and evening collections did not differ ($P > 0.05$) in crude protein (Kirby and Stuth, 1982). Crude protein patterns in masticate of rangeland herbage could be associated with herbage selection rather than diurnal variation.

We found that mean IVDMD increased between 0500 and 1700 h. This was consistent with Fisher et al. (1999), who reported that digestibility was higher in the afternoon than morning for tall fescue hay and cattle masticate. However, digestibility did not differ ($P > 0.05$) in morning and evening-collected cattle masticate in summer rangeland herbage (Kirby and Stuth, 1982). Crude protein and IVDMD were greater in herbage collected from the loblolly pine microsite than the control, confirming previous reports that crude protein and IVDMD increase in herbage with decreasing irradiance (Burner, 2003; Burner and Brauer, 2003; Neel et al., 2001).

Orchardgrass lacked persistence in an unshaded microsite, which would be encountered early in the tree rotation, but thrived in pine microsites later in the tree rotation (Burner, 2003). In the central USA highlands, producers should consider establishing orchardgrass in pine alleys after three to five seasons of tree growth to take advantage of the protective shade. Later in the tree rotation, when shading reduces orchardgrass tillering and mass per tiller (D.P. Belesky, 2003, unpublished data), periodic tree thinning could help maintain the sward. Herbage from this type of silvopastoral system is adequate for a cow–calf operation, although producers should use short periods of rotational grazing and adjustment of stocking rate for sustained production of high quality forage. Relative limitations of irradiance and soil moisture on herbage yield and physiology need further study.

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